PATHOLOGICAL TRANSITION AND FUNCTIONAL VICISSITUDE OF LIVER DURING FORMATION OF CIRRHOSIS BY COPPER

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INTRODUCTION

Numerous investigations have been made regarding cause and mechanism of formation of liver cirrhosis. The same factor has at one time been considered to be a direct, at another to be an incentive cause, with the result that the theories advanced are extremely complicated. The author induced a form of liver cirrhosis by the prolonged administration of copper to rabbits. During this period the histopathological changes occurring in various organs were observed, with special emphasis on the liver, and these were compared with changes in function resulting. The functions of the liver are varied and each is often dissociated; further the liver possesses a high degree of compensatory regeneration, in addition to its having a close relationship to other organs. Hence the nature and degree of its functional disorders are extremely complicated. It is therefore, necessary to make repeated examinations of its function with due attention to other organs intimately related to it, before a correct understanding can be obtained.

METHODS

10 ml of a 1% solution of copper sulphate was given to rabbits orally, daily or every other day. From the start of administration till liver cirrhosis resulted, the macroscopical and histological changes were examined in series. Also at the end of 30 administration, the blood and urinary sugars, santonin detoxication function, serum bilirubin content, total proteins, Takata's reaction, cobalt hydrochloride reaction, urinary urobilinogen tests were made. Histological studies were made by single staining with hematoxylin and double staining by hematoxylin-eosin. For fructose-loading tests in whole blood and quantitative estimation of urinary sugar, after the first bleeding 1 g of fructose per kilogram bodyweight was injected intravenously as a 20% solution, and bleedings made at intervals of 20 minutes for the first hour and at 30 minutes' intervals during the second hour. The total blood sugar was estimated by the method of Hagedorn-Jensen, and the rise in total blood sugar, the maximum rise in blood sugar, the blood sugar quotient, the time of return of hyperglycemia to pre-loading level were obtained. The urinary sugar was estimated at the end of the first and second hours, for total sugar excreted by the Pavy-Kumakawa-Suto method.

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Santosol loading test was made by the intravenous injection of 0.2 ml of 10% santosol per kilogram bodyweight. The time of first appearance of santonin dye was obtained, while at the ends of 3 and 6 hours after administration the total excreted was estimated by the Takasugi-Miyamoto method.

The serum bilirubin was examined by Meulengracht's method, the total protein by the copper sulphate method and the Takata's reaction and urinary urobilinogen by known methods.

RESULTS

In summarising the macroscopic appearances there are no marked changes in the early stage, during the intermediated stage there is seen a slight coloring and the formation of minute granules on the surface, while in the late stage there results hardening of substance, a marked irregularity with uneven surface and the picture of cirrhosis.

Histological changes seen in the early stage were a high degree of degeneration in the liver cells, an instability in the intermediate and an atrophy with compensatory hypertrophy in the late stage. In the early stage degeneration of the hepatic cells manifests itself in an increase of the protoplasm especially in the center of the small lobe. There are seen in the protoplasm small vacuoles and large numbers of small granules. In some cases the nuclei were poor. In cells showing hypertrophy or atrophy the nuclei also showed morphological changes. In the intermediate stage this condition was instable and there were cells with morphological changes but with good nuclear staining, cells with two or more nuclei, indicating some degree of stimulation. In the late stage the cells in the margin of the small lobe were generally enlarged while those in the center showed atrophy. The formation of vacuoles in the proloplasm was marked in cells along the margin of the small lobe. In other words cells that change morphologically by dilution, granule or vacuole formation, later become atrophied or hypertrophied with resulting irregularity in size of the hepatic cells. This formation of jrregularity in size is not due to an alternating atrophy or hypertrophy of tissues and in the small lobe both these stages may be seen in any one portion. Although in the early stages the stellate cells show slight enlargement, the same as the hepatic cells, in the later stage they regain their original morphology and there was no case of any marked proliferation of these cells. Hemorrhage or necrosis did not occur in all cases. Next in all cases examined, including those in the earliest stages (those after 33 days) up to those fed for over the longest period (up to 400 days and over), there were seen minute granules in all the cells scattered or in group formation, although it must be admitted that such cells differed somewhat depending upon the stage of degeneration, atrophy or hypertrophy existing. In the stellate cells there were also seen such granules in abundance in many cases but with the return of these cells to their normal forms and stability such granules could no longer be seen in these cells.

In the interhepatic tissues after a short administration for 180 days or less there is seen a low degree of diffuse or local infiltration by round cells, but after 200 days there was marked infiltration by round cells and proliferation of con-

nective tissue. After 300 days the proliferation of connective tissues becomes further marked, much more than the infiltration by round cells. These round cells are mainly lymphocytes, with some eosinophyles, plasma cells, and megakaryocytes. Polymorphoneutrophiles are seen only unusually. The connective tissue proliferates widely in the interstices and have no special relationship with blood vessels and biliary canals. They surround the small lobe or they may be divided into several parts, each enclosing within itself liver cells. The proliferation of connective tissues is comparatively marked around the border of the liver and connected with the thickening of the liver capsule, The above cellular infiltrations and proliferations connective tissue are most marked in the interstices and there were practically no cases where the hepatic cell cords of the small lobe were involved.

After administration of copper acetate for 30-60 days. *i.e.* at the stage when the hepatic cells begin to degenerate, there manifests dys-function of the sugar metabolism. After 90 days' administration, *i.e.* the stage when the histological degeneration reaches an unstable stage, there results a compensatory action to overcome this lowering in sugar metabolism and there is hence a temperary recovery. However after 120-150 days' with the end of this unstable stage there is seen again a dysfunction of sugar metabolism, with the breakdown of the compensatory action. This functional failure on the part of sugar metabolism continue throughout the precirrhotic to the cirrhotic stages. In other words the results of total blood sugar estimations after intravenous injection of fructose agree with the pathological picture of the liver and present good indications of the organic changes in the liver. No recognizable results were available from urinary sugar estimations.

The excretion of santonin dyes in the urine becomes marked during the degenerative stage. During the early part of the unstable stage when compensatory activity is seen, the increased excretion slighty falls but with the end of the compensatory stage there is seen again increased excretion. During the precirrhotic throughout the cirrhotic stages there is a gradual increase in excretory activity. There were no recognizable changes in examinations of first appearance of the dye. Despite the histological changes in the liver there is an increasing excretion of santonin dyes in the urine, but at present no satisfactory explanation can be made to explain this phenomenon.

The serum bilirubin content showed no changes throughout and the histological changes in the liver warranted no cause for the appearance of jaundice.

Total serum proteins also showed no changes throughout. The Takata's Reaction was negative. The cobalt chloride reaction showed a right-sided reaction with the beginning of marked proliferation of interstitial connective tissues in the liver and this reaction became gradually more marked with the progress in the pathological picture after further administration of copper.

The urnary urobilinogen was in most cases negative throughout, though at times there were cases when it became temporary positive but not specially in order. Hence the urinary urobilinogen qualitative test cannot be considered to be a delicate one for the examination of liver dysfunction.

SUMMARY

In rabbits administered orally with 10 ccm of 1% copper sulphate solution daily or every other day, the most characteristic changes are seen in the liver where there is seen the deposition of granules of a golden color and those irregularly stained by hematoxylin. The liver may be considered to be the chief organ affected. In the early stages after administration of copper the liver cells become turbid, dilate, form vacuoles and show marked degeneration with inflammation of the interstitial tissues. In the intermediate stage there results instability in degeneration of the liver cells, with infiltration by round cells of the interstices and proliferation of the connective tissues. In the final stage the hepatic cells show regressive degeneration with progressive degeneration in certain parts. There is also a marked proliferation of the interstitial connective tissues with a resulting picture of liver cirrhosis. However, this proliferation differs somewhat from that seen in human liver cirrhosis.

In the early stage after copper administration, *i.e.* in the period of hepatic cell degeneration, there results a noticeable dysfunction in sugar metabolism, but during the intermediate unstable stage there is a return to normal of this function. However, with the end of this stage, there appears again a dysfunction and this continues throughout the pre-cirrhotic and cirrhotic stages. No regular results were obtainable from urinary sugar tests.

The excretion of santonin dyes in the urine increased markedly during the early stages of degeneration, became temporarily decreased during the early part of the unstable stage, but again increased with the end of that stage and continues so throughout the pre- and cirrhotic stages. There was no regularity in the time of first appearance of this dye in the urine.

No marked changes were recognized throughout in serum bilirubin and total serum proteins.

The Takata's Reaction was negative throughout. The serum cobalt chloride reaction was unchanged in the early stage *i.e.* the stage of degeneration, but in most cases with the proliferation of interstitial connective tissues in the unstable stage, a right-sided reaction was seen and this became gradually more marked with progress in the pathological changes.

The urinary urobilinogen was negative throughout but sometimes there appeared a temporary positive reaction, without any order.

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EXPLANATION OF FIGURES

FIG. 1. Macroscopic appearance. Rabitt No. 20. Liver. Copper administration 120 times. After 225 days. The liver is dark brown with formation of minute granules on the surface.

FIG. 2. Macroscopic appearance. Rabitt No. 3. Liver. Copper administration 210 times. After 425 days. The liver shows a gross granular surface (liver-cirrhosis).

FIG. 3. Macroscopic appearance. Rabitt No. 3. Liver, reverse side. Copper administration 210 times. After 425 days. The liver shows marked gross granular surface (liver-cirrhosis).

FIG. 4. Microscopic appearance. Rabitt No. 20. Liver. Copper administration 120 times. After 225 days. Single staining with hematoxylin. There are seen in the liver-cell minute brown granules.

FIG. 5. Microscopic appearance. Rabitt No. 3. Liver. Copper administration 210 times. After 425 days. Single staining with hematoxylin. There are seen in the liver-small-lobe local cell-infiltration. Liver-cell shows marked degeneration.

FIG. 6. Microscopic Appearance. Rabitt No. 11. Liver. Copper administration 180 times. After 322 days. Double staining with hematoxylin-eosin. The liver-cell shows irregularity in size. There are seen in the interhepatic tissues a marked cell-infiltration.

FIG. 7. Microscopic appearance. Rabitt No. 10. Liver. Copper administration 180. times After 315 days. Double staining by hematoxylin-eosin. There are seen in the center of the liver-small-lobe degeneration and vacuole formation; in the margin of the liver-small-lobe cell-infiltration.

FIG. 8. Microscopic appearance. Rabitt No. 3. Liver. Copper abministration 210 times. After 425 days. Single staining by hematoxylin The interhepatic tissues begin to proliferate as branches. There is cell-infiltration.

FIG. 9. Microscopic appearance. Rabitt. No. 11. Liver. Copper administration 180 times. After 322 days. Single staining with hematoxylin. There is marked cell-infiltration in the interhepatic tissues, that radiate. The liver-cell shows irregularity in size.

FIG. 10. Microscopic appearance. Rabitt No. 9. Spleen. Copper administration 180 times. After 393 days. Single staining with hematoxylin. There is marked congestion of blood, and numerous blood pigment. There is no change in the folliculi.

FIG. 11. Microscopic appearance. Rabitt No. 5. Bone marrow. Copper administration 210 times. After 479 days. Single staining with hematoxylin. There are numerous gigantic-cells, and gross hematoxylin granules in the reticuloendothelial-cell.

FIG. 12. Microscopic appearance. Rabitt. No. 11. Pancreas. Copper administration 180 times. After 322 days. Double staining by hematoxylin-eosin. Langerhans-islands are large and part of them show the structure of an adenoma. There is no change in the gland-tissue.

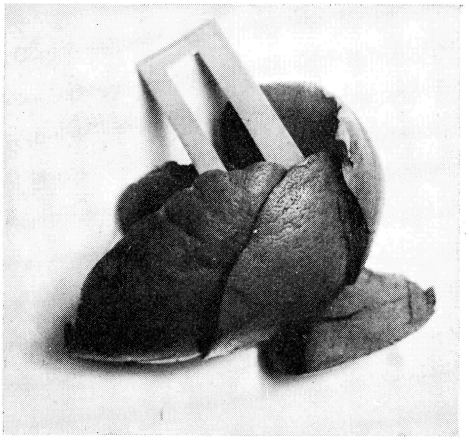

FIG. 1

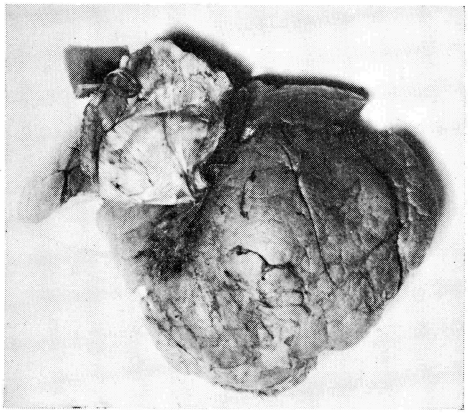

FIG. 2

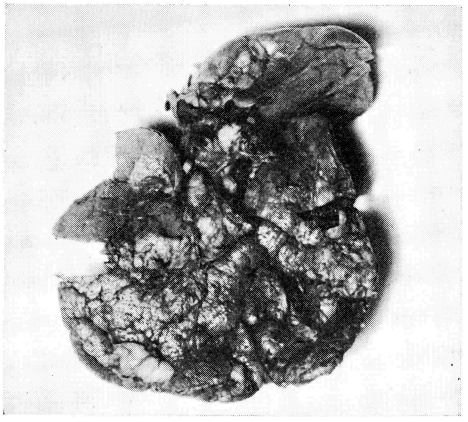

FIG. 3

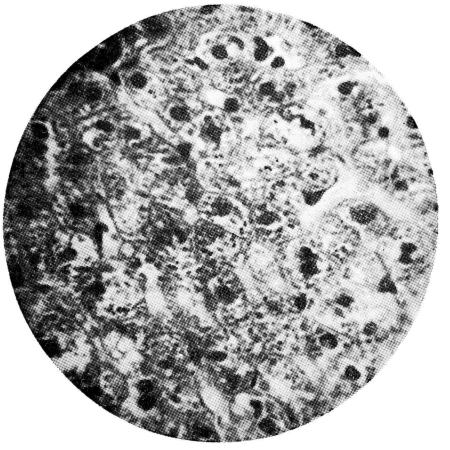

FIG. 4

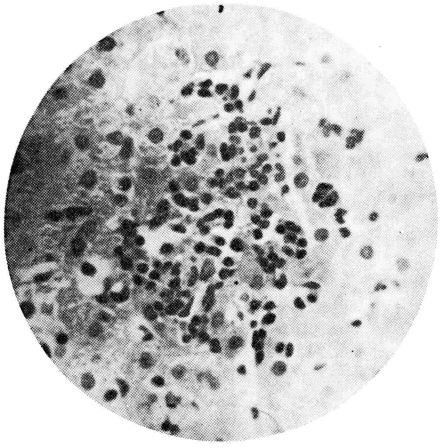

FIG. 5

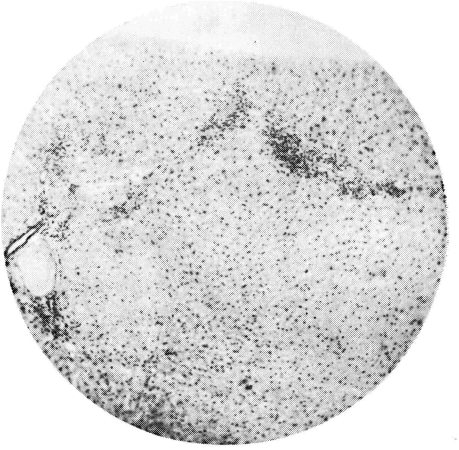

FIG. 6

Katsuui Tachibana: *Pathological Transition and Functional Vicissitude of Liver during Formation of Cirrhosis by Copper.*

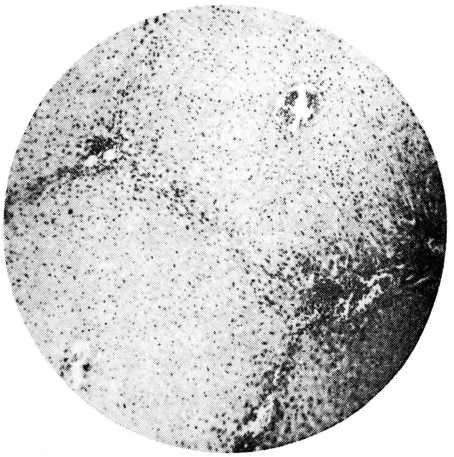

FIG. 7

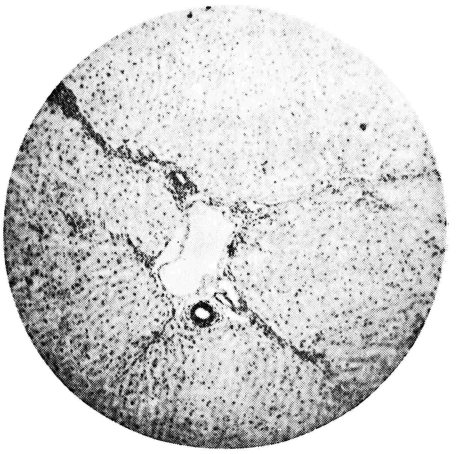

FIG. 8

FIG. 9

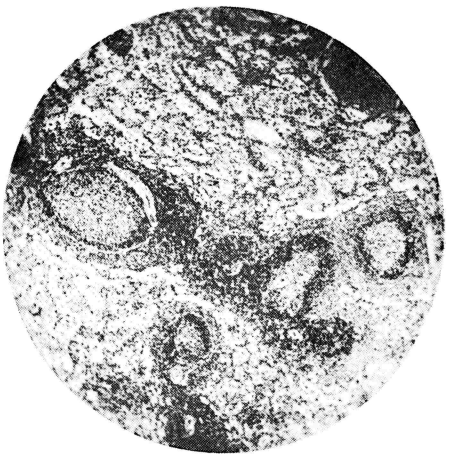

FIG. 10

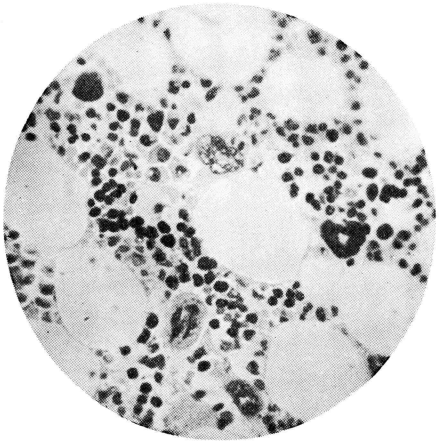

FIG. 11

FIG. 12